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Separation of Homologous Series of Dinitrophenyl Amines by Thin-Layer Partition Chromatography

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INTRODUCTION

Separation of an homologous series of dinitrophenyl (DNP) amines by thin-layer chromatography (TLC) has not been documented to date. This elegant technique offers the advantages of both speed and sensitivity over paper and column chromatography, and its use is fast-becoming a standard laboratory procedure. The present manuscript describes a thin-layer partition chromatographic system for separating a homologous series of DNP primary amines from C_1 through C_{10} in about 1 hour. The same system will separate the first five members of symmetrical secondary DNP-amines.

It has been found possible to detect smaller amounts of the DNP-amines over that detectable by the natural yellow color by spraying the finished plate with a mixture of alcoholic KOH and nitromethane. Visual detection of 10^{-3} μmole is then readily accomplished.

It was also found possible to convert 10^{-3} μmole of a parent amine to the DNP derivative at the origin of a thin-layer plate without altering the mobility of the DNP-amine prepared in this manner with respect to the authentic derivative.

MATERIALS

"Deactivated" Seasorb 43. Seasorb 43 (Fisher Scientific Co.,[1] Silver Spring, Maryland) is fully deactivated with respect to its ability to adsorb DNP-amines by heating it in a muffle furnace at 800° C for 16 hours.

The Research Specialties TLC equipment was utilized except for the tank, which was the same as that used by Schwartz and Parks (*3*).

[1] Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

DNP derivatives were prepared by using a twofold molar excess of the amine to 1-fluoro-2,4-dinitrobenzene (FDNB) in methanol and recrystallizing solid derivatives from MeOH. Liquid derivatives were washed with 1 *N* HCl, water until neutral and then with 80% methanol followed by vacuum desiccation.

METHODS

Preparation of plates. Fourteen g of "deactivated" Seasorb 43 and 7 g of Celite 545 are added to 8 ml of polyethylene glycol 400 (the stationary phase) and 48 ml of 95% EtOH in a stoppered 125-ml Erlenmeyer flask. The flask is shaken vigorously by hand for 5 minutes and then spread in the usual manner over five 8 × 8-inch plates. The plates are air-dried for 15 minutes and then dried for 10 minutes at 100° C. The plates are then ready for use or they may be stored in an air-tight, moisture-free desiccator over $CaCl_2$.

Development of plates. The bottom $\frac{1}{4}$ inch of support is scraped from the plate with a straight edge, and the DNP-amines are spotted from a methylcyclohexane solution or ((in the case of DNP-methylamine) from a methyl cyclohexane-ethyl acetate solution. The spotted plate is then developed (in the direction of application of the slurry) with *n*-heptane saturated with polyethylene glycol 400 in a tank lined with filter paper and which has been equilibrated for at least 16 hours. At the end of the development (about 1 hour) the plate is removed, the solvent is evaporated, and the plate is sprayed with a 1:1 mixture of 0.5 *N* ethanolic KOH and nitromethane, mixed immediately before use. Deep pink to lavender spots develop within a minute. The color fades back to the original yellow hue within 30 minutes.

Preparation of DNP derivatives on the plate. Conversion of the parent amine to the DNP derivative at the origin of a thin-layer plate was effected by spotting a 5-fold (molar basis) excess of FDNB in benzene at the origin and overspotting with the amine in benzene solution followed by development of the plate as described above.

RESULTS AND DISCUSSION

Photographs of typical separations of DNP-primary and symmetrical DNP-secondary amines are shown in Figs. 1 and 2, respectively. The separation of the first 10 members of primary amines and first 5 members of secondary amines is practically linear with respect to chain length. Impregnation of more or less stationary phase gave poorer separations that

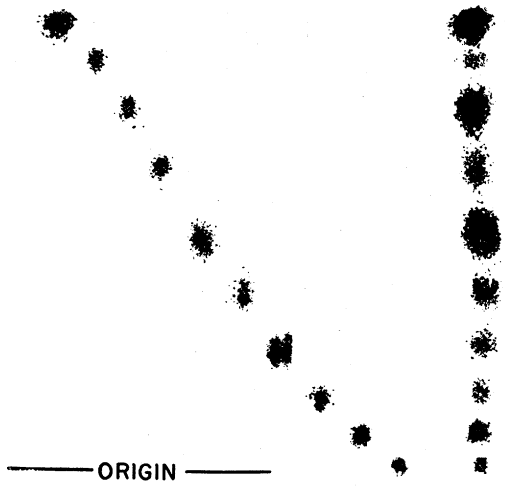

FIG. 1. Separation of dinitrophenyl amines by thin-layer partition chromatography. Diagonally from top to bottom: decyl amine through methyl amine. Column on right represents a mixture of all 10 amines.

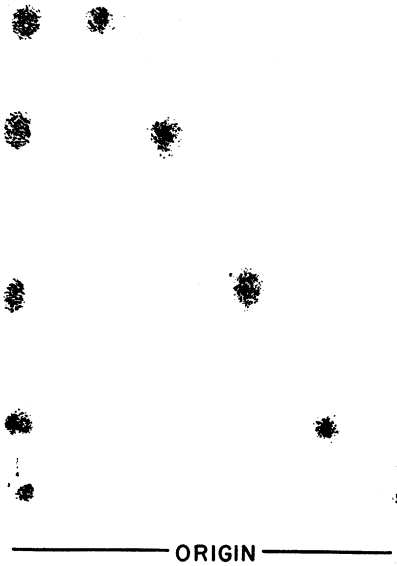

FIG. 2. Separation of dinitrophenyl amines by thin-layer partition chromatography. Diagonally from top to bottom: di-*n*-amyl amine through di-*n*-methyl amine. Column on left represents a mixture of all 5 amines.

resulted in S-shaped or inverted S-shaped curves. No difficulty was encountered in obtaining straight fronts when the stationary phase was incorporated in the slurry as described. This was not the case when the stationary phase was impregnated on the plate by dipping the plate in an acetone solution of polyethylene glycol 400. Badings and Wassink (*1*) used an analogous technique for TLC partition chromatography of 2,4-dinitrophenylhydrazones.

Use of a nitromethane-alcoholic KOH solution for coloring the spots has not been described to the authors' knowledge, although use of dimethylformamide in conjunction with alcoholic KOH has been tested on many dinitro compounds (*2*). Use of alcoholic KOH alone gave no intensification of the spots.

Through the use of nitromethane-alcoholic KOH spray, 10^{-3} μmole of a DNP-amine is readily detected. Colors with dimethylformamide-alcoholic KOH were of a different hue and appeared to be less intense.

Preparation of submicrogram amounts of DNP-derivatives of amines at the origin of a plate followed by chromatography should be a useful adjunct to microanalyses of biological systems as well as a possible confirmatory technique for suspected amine peaks emerging from a gas chromatograph. Spotting 10^{-3} μmole of the amine still gives a readily visualized spot on the completed chromatogram. The reaction on the plate probably runs very well because of the basicity of the magnesia, although it was not ascertained whether the reaction goes to completion. Excess FDNB, and any artifacts which may be produced in the reaction, do not move off of the origin and thus do not interfere with any of the DNP-amine spots except methylamine.

All of the amines used in the study are commercially available. It is recommended that knowns be run simultaneously in the investigations of an unknown.

SUMMARY

A thin-layer partition system is described for separating the first 10 members of the dinitrophenyl derivatives of *n*-primary and the first five members of symmetrical secondary amines. Polyethylene glycol 400 and heptane are used as the stationary and mobile phases, respectively. A mixture of Celite 545 and deactivated magnesia is used as the support. Complete separation is effected in about 1 hour. Spraying the finished plate with a mixture of alcoholic KOH and nitromethane gives pink to lavender spots which permit the detection of 10^{-3} μmole. A procedure is also described for the preparation of as little as 10^{-3} μmole of a dinitrophenyl amine at the origin of a chromatoplate.

REFERENCES

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